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FINAL REPORT ON BIOLOGIC TESTS
OF SAMPLES 22260p5 and 22263p2.

Report submitted to : Construction Products Division
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INTRODUCTION 03656863

After experimental deposition of certain preparations of asbestos or other mineral fibers into the pleural space, granulomatous, fibrotic and neoplastic lesions have been described in hamsters¹⁻⁸ and in rats.^{9,10} Development of such lesions has been associated with geometry of fibers in preparations that yielded positive results.^{3-7,9,10} The present report describes an experiment in which a specially milled preparation of tremolite, and a mixture of that preparation with vermiculite, were deposited in the pleural space of hamsters. Granulomatous, fibrotic and neoplastic lesions were observed. Significance of the findings is discussed.

METHODS

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Materials. All samples were prepared by Construction Products Division, W.R. Grace & Company. These samples were identified as: (1) Tremolite (22260p5) and (2) 50% tremolite + 50% vermiculite (22263p2). The sample of tremolite will be referred to as Sample 60, and the tremolite-vermiculite mixture as Sample 63. The preparation of tremolite was the same in both samples.

Sample 60 had been submitted to Arthur D. Little, Inc. for measurement of fiber size by scanning electron microscopy at 2000X. Geometric mean length was reported as 2.07 μm , geometric mean diameter as 0.2 μm , average aspect ratio 10.36. Of 125 fibers measured, 16 (12.8%) were longer than 5 μm .

The samples were received in our laboratory as dry powders. Sample 60 was light gray in color, Sample 63 was a darker gray. For injection into animals, aliquots of the samples were suspended at a concentration of 25 mg per 0.5 ml in 0.9% NaCl solution in demineralized water and sterilized in an autoclave at 250°F for 15 minutes. Suspensions of each sample dispersed readily on shaking, but immediately before injection into animals the suspensions were sonicated for 10 minutes in a Bransonic 12 (Branson Instrument Co., Stamford, Conn.). In the course of injecting animals, suspensions were agitated by repeated pipetting.

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Animals. Male hamsters of the Lak:LVG strain were obtained from Charles River Laboratories, Lakeview, NJ, the colony that provided all hamsters used in our previously reported experiments.¹⁻⁸ The animals were received in our laboratory at about 6 weeks of age. They were held for observation for 2 weeks before start of tests. The animals were housed in mesh-bottom stainless steel cages and provided ad libitum with water and Purina Rodent Laboratory Chow (Ralston Purina Co., St. Louis, MO.). Lighting was controlled to give 12 hours of light (6AM to 6PM). Temperature and humidity were held at $21 \pm 2^{\circ}\text{C}$ and $45 \pm 5\%$ RH respectively. Animals in each treatment group were tattooed on the abdomen with their group number and identified individually by ear punch.

Design and Procedures. The experiment compared pathologic findings in 3 groups of 70 male Lak:LVG hamsters each injected at the age of about 2 months. Injections were made via a 20 gauge needle into the right pleural cavity. Hamsters in Group 1 each received 25 mg Sample 60 suspended in 0.5 ml 0.9% NaCl. Hamsters in Group 2 each received 25 mg Sample 63 suspended in 0.5 ml 0.9% NaCl. Hamsters in Group 3 each received 0.5 ml 0.9% NaCl.

The design was to kill 10 hamsters from each group at 3 months for pathologic examination for early lesions, and to maintain all others for long-term tests for oncogenicity. In the first 3 months, an outbreak of acute enteritis (a common spontaneous disease of hamsters) resulted in deaths: 15 in Group 1, 19 in Group 2 and 22 in Group 3. Additional hamsters were treated and added to the 3 groups to replace these losses.

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Pathology: Except for a few eaten or missing animals (1 in Group 1, 1 in Group 2, 3 in Group 3), all animals were examined by necropsy. At necropsy, gross findings were recorded. All tumors were fixed in formalin and sections were cut and stained with hematoxylin-eosin for histopathologic diagnosis. Sections of lungs and other tissues from representative animals were cut and stained with hematoxylin-eosin. Sections of lungs from hamsters killed 92 days after treatment were also stained with a modified Gomori's One Step Trichrome Method using aniline blue (11).

Electron microscopic studies were made on selected tissues. For electron microscopy, tissues were fixed in paraformaldehyde, embedded in Spurr's epon mixture, and thin sections were examined in a Hitachi HU 12 electron microscope.

Histopathologic and electron microscopic studies were made by our consultant in pathology, Harold J. Sobel, M.D.

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RESULTS

Observations on materials. A small amount of each sample was suspended in water and drops were examined by phase microscopy with a 40X objective and 10X ocular on a slide with a 1 mm scale graduated in 10 μm divisions.

Sample 60: Essentially all particles seen with this optical set up are fibers about 5 to 15 μm in length. There are occasional longer fibers up to about 30 μm long. All seem to have parallel sides, resembling fibers rather than shards. There are rare amorphous, irregularly shaped, roughly round yellowish particles about 5 to 15 μm in diameter.

Sample 63: Same fibrous particles as in Sample 60 plus numerous amorphous irregularly shaped roughly rounded yellowish particles about 5 to 15 μm in diameter.

Suspensions autoclaved for injection of animals were allowed to stand overnight at 4°C. Examination of autoclaved suspensions by phase microscopy showed no change in the fibrous particles in either sample, but the amorphous yellow (vermiculite) particles appeared to have approximately doubled in size. Before autoclaving, the amorphous yellow particles appeared to be aggregates of platy crystals. After autoclaving, these aggregates appeared less tightly compacted.

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Findings in animals.

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At necropsies of animals examined between 70 and 89 days after injection with control saline, no remarkable changes were found in lungs or pleura, whereas pleural adhesions were seen in all of 10 hamsters examined 49 to 92 days after injection of Sample 60 and in all of 10 hamsters examined 41 to 92 days after injection of Sample 63. Extent of adhesions was similar in animals treated with one sample as compared to the other (Table 1).

Histological sections were prepared for microscopic examination of lungs and pleura from each of 5 hamsters killed 92 days after treatment with Sample 60 and from each of 5 hamsters killed at 92 days after treatment with Sample 63. In sections stained with hematoxylin-eosin, each of these specimens showed, seated on the pleura, granulomatous lesions composed of macrophages and multinucleated giant cells encompassed by fibrous tissue. Mineral particles were prominent in these granulomas, showing that they were depots of injected material. In areas distant from granulomas, there was fibrous thickening of pleura. Sections stained with a modified Gomori's One Step Trichrome Method using aniline blue (11) showed that thickened pleura in each case was composed of fibrous tissue that stained blue, indicative of collagen. There was no impressive difference in extent of granulomatosis or fibrosis in response to Sample 60 as compared to Sample 63.

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Surviving animals were maintained and examined with special reference to incidence of tumors. As a measure of general health, average body weights of hamsters followed for incidence of tumors are shown in Table 2, from which it can be seen that average body weights of Groups 1 and 3 were closely comparable throughout the experiment, but that average body weight of Group 2 tended to run somewhat higher. The number of long-term survivors (133 days or longer) was 58 in Group 1, 61 in Group 2 and 60 in Group 3. As listed in Table 3, most of these animals died of natural causes, a few were killed because moribund, and all survivors were killed for examination at 600 to 610 days after injection. Average survivorship after injection was 410 days in Group 1, 445 days in Group 2 and 421 days in Group 3.

Common findings at necropsy were enteritis (19 cases in Group 1, 17 in Group 2, and 23 in Group 3), thrombi in cardiac atria (9 cases in Group 1, 4 in Group 2, and 11 in Group 3), renal disease (8 cases in Group 1, 12 in Group 2, and 7 in Group 3). Enteritis, cardiac thrombi and renal disease are common causes of death in untreated control hamsters (12).

Neoplastic lesions and the times at which they were found are listed in Table 3 and are described individually in Appendices A, B and C. Tumors regarded as attributable to treatment are listed in Table 4. Questions of diagnosis and significance of these tumors are considered in Discussion.

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DISCUSSION

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After intrapleural injection into hamsters of 25 mg doses of a preparation of tremolite (Sample 60), or 25 mg doses of a mixture (Sample 63) of equal parts of Sample 60 with a preparation of vermiculite, granulomatous and fibrotic lesions were found in all animals examined 41 or more days after injection. After 245 days, sarcomatous intrathoracic tumors were found in occasional animals that had been injected with Sample 60 or Sample 63. Also, after injection of Sample 63, there was a subcutaneous sarcoma in the area of injection in one hamster.

Granulomatous, fibrotic and sarcomatous lesions are known to occur in rodents after injection of a wide variety of foreign bodies, such as discs of plastics, ivory, pieces of glass, quartz dust and various metal foils.¹³⁻¹⁷ Such lesions found in the present experiment are attributed to treatment with Samples 60 and 63, since no such lesions were found in control hamsters that had received intrapleural injections of the medium (saline solution) used to suspend the test samples for injection.

Other tumors found in hamsters treated with Samples 60 or 63 are not attributed to treatment, since similar tumors were found in the control group or are known to occur as spontaneous events in hamsters.^{12,18,19,20} Thus, the single pulmonary carcinoma found in this experiment (Appendix A, Hamster 52-4) is not attributed to treatment, since a pulmonary carcinoma has been reported in a control hamster from the colony that provided animals for this experiment.²⁰

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Granulomatous, fibrotic and neoplastic responses to the two samples were not impressively different in extent, frequency or time at which they were found (Tables 1,3,4). This could reflect a plateau of response to the relatively high dose of tremolite used. After intrapleural injection into hamsters of lesser doses of preparations of asbestos fibers, dose-related responses, including no-effect levels, have been found.⁶ Accordingly, the present experiment provides no evidence for oncogenicity of vermiculite. After intrapleural injection of 25 mg vermiculite into rats, granulomatous and fibrotic responses were found, but no tumors resulted in an experiment reported by others.²¹

The nature of intrathoracic tumors found in animals in this experiment, and after intrapleural deposition of other mineral fibers by ourselves and others, is a subject for debate. Surfaces of the pleura and peritoneum are lined by mesothelial cells, and tumors spreading along such surfaces with no other identifiable primary source have been termed mesotheliomas with implication that they arose from mesothelial cells. The term "mesothelioma" has been used as a pathologic diagnosis for such tumors in rats by Wagner.⁹ In early work, Stanton diagnosed such tumors as mesotheliomas, but has subsequently employed the term "pleural sarcomas" in tabulating all tumors resulting from intrapleural deposition of test materials into rats by his group.¹⁰ The majority of such experimentally induced tumors in rats and in hamsters are sarcomatous, whereas tumors diagnosed as mesotheliomas in human beings, though occasionally sarcomatous (mesenchymal), often have

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epithelial or mixed epithelial and mesenchymal characteristics.²²⁻²⁵ Gross²⁶ has challenged propriety of extrapolating data on intrathoracic sarcomas in experimental animals for estimation of potential mesothelioma risks for human beings.

In the present experiment, all intrathoracic tumors attributed to treatment were sarcomatous except for one case in which the cells, though spindle-shaped in the manner of sarcoma cells, did not appear malignant and were regarded as fibrous-type. No tumors resembling epithelial types of mesotheliomas were found.

In studies of histogenesis and ultrastructure of tumors or intrapleural after intraperitoneal injection of asbestos into rats and mice, proliferative lesions of mesothelium were shown to have both epithelial and mesenchymal components.^{27,28} Ultrastructural features associated with mesothelial cells have been described from electron micrographs of epithelial-type mesotheliomas in man.^{23,24} These features include microvilli, epithelial junctions, basement membranes and cytoplasmic filaments. In electron microscopic studies of intrathoracic tumors in hamsters after intrapleural injection of asbestos fibers, we recently reported microvilli, epithelial junctions, basement membranes and cytoplasmic filaments in 3 epithelial-type tumors, and, less prominently, in 2 fibrous and 4 sarcomatous types, supporting a diagnosis of pleural mesothelioma in those cases.⁸

Of the 10 sarcomatous intrathoracic tumors found in the present experiment, 4 were examined by electron microscopy.

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In each of these 4 cases, occasional cells showed microvilli, epithelial junctions (desmosomes), basement membranes and cytoplasmic filaments, supporting a diagnosis of mesothelioma.

(Appendix A, Hamster 8-4; Appendix B, Hamsters 86-5, 5-5, 61-5).

For purposes of description and tabulation, we have used the term "pleural mesothelioma" in Appendix A and B, and in Tables 2 and 4, not only for these 4 cases but also for 6 other cases with comparable gross and histologic characteristics.

In a subcutaneous sarcoma, electron micrographs revealed cytoplasmic filaments but no clearly defined basement membranes, microvilli or desmosomes (Appendix B, Hamster 3-5).

Presence of C-type virus particles was noted in our previous report on electron microscopy of asbestos-induced tumors diagnosed as mesotheliomas in hamsters.⁸ Similar particles were seen in cells of all of the 4 tumors so diagnosed and examined by electron microscopy in the present experiment. The findings of these particles raises a question as to whether a virus may have played a role in genesis of these tumors and perhaps in the general problem of asbestos carcinogenesis. Although some of the observable particles in our previous and present studies appear to be C-type virus particles, others in both studies may be R-type particles of unknown significance.

Electron micrographic studies of the subcutaneous sarcoma (Ap.-B, Hamster 3-5), a lymphoma (Ap. B, Hamster 16-5), an adrenal adenocarcinoma (Ap. C, Hamster 17-3), and normal

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liver, kidney and lymph nodes (Ap. B., Hamsters 78-5 and 79-5) revealed no identifiable virus-like particles in any of these tissues.

Tumor response of hamsters and rats to intrapleural injection of a variety of asbestosiform and other fibrous minerals has been shown to be reduced or eliminated by reduction in lengths of fibers.^{3-7,10} In previous experiments on intrapleural injection of tremolite into hamsters, tumors resulted in a dose-related pattern in response to preparations containing large numbers of fibers in lengths and diameters readily visible by optical (phase) microscopy at 400X, but no tumors resulted from a preparation of tremolite containing relatively few particles identifiable as fibers at 400X.⁷ Subsequent measurements by scanning electron microscopy at 1250X show that the percent of fibers 10 μm or longer is 10 to 17% in the tremolite samples that induced tumors compared to 2% in the preparation of tremolite that induced no tumors. In the preparation of tremolite used in the present experiment, mean fiber length as measured by electron microscopy was 2.07 μm , but numerous fibers longer than 10 μm

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were readily visible by optical (phase) microscopy at 400X. However, we found no tumors in hamsters after intrapleural injection of an industrial talc containing 50% tremolite with numerous fibers longer than 10 μm and readily visible at 400X.^{5,7} Measurements by scanning electron microscopy at 1250X show that 18% of fibers in that sample were longer than 10 μm . Accordingly, mere observation of fibers in certain size ranges is not evidence that a material will be oncogenic.

It should be recalled that granulomatosis and fibrosis followed by development of malignant tumors is known to occur in rodents after injection of a wide variety of foreign substances into subcutaneous tissues or body cavities.¹³⁻¹⁷

Need for caution in extrapolation of data from injection experiments is well illustrated by the fact that malignant tumors have been found after injection of glass fibers into the pleural space of rats, but not after long-continued inhalation exposures of rats to glass fibers.²⁶

Materials injected into the pleural space are not subject to clearance mechanisms that operate in the case of inhaled particles. Results of injection experiments must therefore be regarded as screening data for selection of substances or particle shapes that may merit further study by more elaborate techniques such as intratracheal instillations or inhalation exposures. Thus, the negative results of intrapleural injection of vermiculite constitute good evidence for lack of oncogenicity of vermiculite.²¹ Results of previous experiments⁷ and the present work suggest further studies of tremolite fibers in certain size ranges with attention to amounts of exposures.

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SUMMARY

Male Lak:LVG hamsters about 2 months of age were given intrapleural injections of 25 mg of a preparation of tremolite or 25 mg of a mixture of equal parts of that preparation with vermiculite. For injection, the materials were suspended in 0.9% NaCl solution in water. Control hamsters were given intrapleural injections of the suspending medium.

The animals were observed to a maximum of 610 days after injection and were necropsied. Granulomatous and fibrotic lesions were found on the pleura of all animals necropsied 41 days and subsequently after injection of the two samples of minerals. Intrathoracic tumors considered to be mesotheliomas were found in occasional animals that had been treated with either sample. The first tumor considered to be a mesothelioma was found 245 days after injection. No granulomatous or fibrotic lesions of the pleura and no mesotheliomas were found in the control animals. There was no impressive difference in pathologic responses to the preparation of tremolite as compared to the mixture of that preparation with vermiculite.

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Table 1. Rating of Lungs for Pleural Adhesions.

0 = no adhesions

1 = minimal adhesions

Sample 60

4 = extensive adhesions

Hamster No.	No. days after injection	Rating of adhesions	Hamster		No. days after injection	Rating of adhesions	Hamster		No. days after injection	Rating of adhesions
			No.	Rating of adhesions			No.	Rating of adhesions		
13	92	4	13	92	3	49	89	0	0	0
14	92	4	30	92	4	37	84	0	0	0
15	92	4	44	92	4	35	79	0	0	0
17	92	4	45	92	4	13	75	0	0	0
18	92	4	47	92	4	11	74	0	0	0
30	81	2	77	62	3	20	71	0	0	0
76	67	3	27	43	3	15	.71	0	0	0
68	49	3	24	42	4	16	71	0	0	0
73	49	3	28	42	4	18	71	0	0	0
77	49	2	26	41	3	46	70	0	0	0
Average		3.3				3.6				

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Table 2. AVERAGE BODY WEIGHTS IN GRAMS OF HAMSTERS IN TESTS FOR
CARCINOGENICITY AT INTERVALS IN DAYS (± 15) AFTER INJECTION.

Group	Start	100	200	300	400	500	550
		days	days	days	days	days	days
1	92	151	154	165	160	157	150
2	92	152	164	170	171	163	154
3	92	147	149	159	159	152	154

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Table . SURVORSHIP AND LIST OF TUMORS . E. A number refers to an individual hamster and is the length of life in days after injection.

Group 1 (Sample 60)

Day	Tumor	Day	Tumor
133		428	
133		445	
147		457	
K174		467	
181		497	
185		X505	RABDOMYOSARCOMA
NE198		K508	Adrenal adenoma
218		512	MESOTHELIOMA
220		K514	MESOTHELIOMA
221		516	
245	MESOTHELIOMA	522	
252		525	
283		K526	
290		527	
300		K533	NNM338
320		535	
324		K547	
325		555	
326		564	
336		K570	MESOTHELIOMA
338		574	SALIVARY CA + ADRENAL ADENOCA
344		K577	
353			
359		596	MESOTHELIOMA
361	MESOTHELIOMA	KE601	
375	Adrenal adenoma	KE601	HEPATOMA
400		KE601	
413	PULM. ADENOCA	KE601	
414		KE602	
		KE602	
		KE602	

Group 2 (Sample 63)

Day	Tumor	Day	Tumor
133		140	
133		148	
147		176	
K174		178	RHABDOMYOSARCOMA
181		183	
185		187	
NE198		207	
218		225	
220		234	
221		310	
245	MESOTHELIOMA	312	
252		323	
283		329	
290		337	
300		NNM338	LYMPHOMA
320		339	
324		345	ADRENAL ADENOCA
325		369	SUBCUT. SARCOMA
326		372	
336		375	THYROID CA
338		K391	MESOTHELIOMA
344		399	Adrenal adenoma
353		401	Adrenal adenoma
359		K405	
361	MESOTHELIOMA	KE601	
375	Adrenal adenoma	KE601	
400		KE601	
413	PULM. ADENOCA	KE601	
414		KE602	
		KE602	
		KE602	

For control Group 3, see table 3 continued.

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Table 3 (continued)

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Group 3

Saline Control

Day	Tumor	Day	Tumor
135		482	
135	NNE	486	
145		492	
145		496	NNM
150		497	
154		500	
156		517	
165		524	
167		525	Early SQUAMOUS CELL CARCINOMA, forestomach
175		547	
182	K	553	
188		562	
204	MALIGNANT MELANOMA	574	
223		581	ADRENAL ADENOCARCINOMA + Adrenal adenoma
228		585	
259		595	
291		600	KE
306		601	
315		601	KE HEPATOMA, adrenal adenoma
372		601	KE ADRENAL ADENOCARCINOMA
385		601	KE
387		602	KE ADRENAL ADENOCARCINOMA
393		602	KE Splenoma
414	Adrenal adenoma	602	KE
427		602	KE Adrenal adenoma
454		602	KE
460		602	KE
477		610	KE
477		610	KE
480		610	KE

NNE = no necropsy because eaten

NNM = no necropsy because missing

K = killed because moribund

KE = killed to terminate experiment

Malignant tumors are listed in CAPITAL LETTERS.

Benign tumors are listed in small letters.

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Table 4. Tumors attributed to treatment.

<u>Time after injection</u>	<u>Hamster Number</u>	<u>days</u>	<u>Tumor diagnosis</u>
Group 1 (Sample 60)			
245	35-4		Pleural mesothelioma, sarcomatous, with metastases
361	43-4		" " "
514	8-4		" " "
570	26-4		" " , With metastases
597	57-4		" " "
Group 2 (sample 63)			
183	38-5		Intrathoracic rhabdomyosarcoma
391	86-5		Pleural mesothelioma, sarcomatous
405	5-5		" " " , With metastases
450	61-5		" " "
518	67-5		" " "
545	82-5		" " "
588	3-5		Subcutaneous sarcoma, right chest wall

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Appendices A, B and C.

NECROPSY REPORTS ON TUMOR-BEARING HAMSTERS

Each case is identified by the number of the individual hamster and by the number of days between injection and necropsy. Preceding the number of days, the letter K signifies that the animal was killed because moribund. Animals not so designated were found dead.

All animals in Groups 1 and 2 showed scattered foci of yellowish brown material on the right pleura and often on the left pleura and/or pericardium. In these necropsy reports, such lesions are referred to as "depots" since histological examination of them showed collections of the injected mineral particles surrounded by or included within macrophages and multinucleated giant cells. In these reports, such "depots" are distinguished from other pleural adhesions, which were diffuse gray lesions that on histologic examination showed fibroblastic cells.

Appendix A (pages A-1 to A-9) contains reports on animals treated with Sample 60 (Group 1).

Appendix B (pages B-1 to B-17) contains reports on animals treated with Sample 63 (Group 2).

Appendix C (pages C-1 to C-9) contains reports on control animals treated with suspending medium (Group 3).

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Appendix B.

GROUP 2.

Hamster No. 38-5. 183 days.

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Pleural adhesions extend over all surfaces of right and left lungs. Depots lie between right lower and right middle lobes, between infracardiac lobe and left lung, between infracardiac lobe and diaphragm, and in subcutaneous tissue of right chest. A mass of solid tumor ($25 \times 20 \times 20$ mm) lies in the right costophrenic angle. Another such mass ($22 \times 15 \times 12$ mm) is in the left costophrenic angle. A left mediastinal node is enlarged ($6 \times 4 \times 3$ mm).

Histopathology: Normal lymph node. Foci of fibrotically thickened pleura with crystalline material within and without phagocytic cells including multinucleated giant cells.

Surrounding, compressing and invading the lung as well as infiltrating nearby striated muscle of chest wall or diaphragm is a malignant tumor composed of oval, pink cells, often tadpole or strap forms with a fibrillar cytoplasm. There are large numbers of multinucleated giant cells. The nuclei are pleomorphic. There are foci of necrosis.

Diagnosis: Rhabdomyosarcoma.

15057984

B-2

Hamster No. 86-5. K 391 days.

Pleural adhesions over all lobes of right lung. Scattered depots in right pleura and over heart. A tumor (42 x 20 x 20 mm) fills left chest.

03656888

Histopathology: Lung surrounded by tumor composed of compact masses of elongated cells with large vacuolar nuclei. There are 1 to 3 mitoses per high power field. The tumor invades the lung and adjacent muscle of chest wall or diaphragm. Within the tumor there are depots of crystalline fibers. There is a metastasis in a lymph node.

Electron microscopy of tumor: Nuclear irregularity. Large nucleoli. Some cells show tight junctions, desmosomes, microvilli, basement membranes, filaments in cytoplasm. C particles are noted in endoplasmic reticulum.

Diagnosis: Pleural mesothelioma, sarcomatous type.

15057985

B-3

Hamster No. 74-5. K 423 days.

03656889

Moderate pleural adhesions and depots in right and left chest. There is an area 8 x 6 x 5 mm area of firm white tissue lying between right upper and right middle lobes. Liver is enlarged and has white foci.

Histopathology: Chest organs have depots and non-tumorous atypical foci in muscle, with muscle degeneration and proliferation of sarcolemma. Liver shows a small hepatoma and congestion.

Diagnosis: Hepatoma.

15057986

B-4

03656890

Hamster No. 5-5. K 405 days.

Pleural adhesions over all lobes of right lung and between mediastinum and left lung. A few small depots on right pleura. A mass 21 x 18 x 12 mm fills left costophrenic angle. Tissue resembling this tumor found at apex of left lung, over right upper lobe, on cut surface of right lower lobe, in liver surrounding gall bladder, surrounding stomach and spleen, and on peritoneum.

Histopathology: Left lung surrounded by tumor composed of solid masses of oval and spindle-shaped cells with large vesicular nuclei and big nucleoli. The tumor contains depots of crystalline fibers. There is invasion of the lung and many metastatic deposits in vessels within the lung. Right lung is surrounded by similar tumor cells and contains metastases. Nodules of similar tumor cells in liver, kidney, around stomach and infiltrating muscle of abdominal wall.

Electron microscopy on tumor: Nuclear irregularity. Large nucleoli. Prominent mitotic activity. Some cells show tight junctions, and/or desmosomes, microvilli, basement membranes, cytoplasmic filaments. Prominent C particles in endoplasmic reticulum.

Diagnosis: Pleural mesothelioma, sarcomatous type, with metastases.

15057987

B-5

Hamster No. 61-5. K 450 days.

03656891

Pleural adhesions and depots around all lobes of right lung. A mass ($40 \times 30 \times 15$ mm) fills left chest and is adherent to chest wall and diaphragm. Incisions of this mass reveal left lung surrounded by tumor. There are nodules of tumor tissue on right side of peritoneal surface of diaphragm. In mesentery there are 3 well separated tumor nodules that are 4, 6 and 8 mm in diameter. Peritoneal surface, including tunica vaginalis testes, is otherwise not remarkable. There is an enlarged (6 x 4 3 mm) pararenal node. Other organs not remarkable..

Histopathology: Lung surrounded by tumor composed of plump or slightly elongated cells with large vesicular nuclei and prominent mitotic activity. Pancreas with mass of similar tumor. Metastases of identical tumor in paraadrenal lymph nodes.

Electron microscopy of tumor: Nuclear irregularity. Large nucleoli. Prominent mitotic activity. Some cells show tight junctions, and/or desmosomes, microvilli, basement membranes, cytoplasmic filaments. Prominent C particles in endoplasmic reticulum.

Diagnosis: Pleural mesothelioma, sarcomatous type, with metastases.

15057988

B-6

03656892

Hamster No. 67-5. K 518 days.

Depots seen anteriorly between right lung and chest wall, between heart and diaphragm, in pericardium, and between left lung and chest wall. Pleural adhesions extend over all lobes of right and left lungs. In left costophrenic angle, there is a 14 x 12 x 11 mm soft mass.

Histopathology: At base of lung there is a tumor composed of large plump cells with large vesicular nuclei. In some areas, the tumor cells are elongated. The tumor has invaded lung. There are deposits of these tumor cells in a pulmonary vessel and in a mediastinal node. Several mediastinal nodes contain many crystalline fibers. There are a few small areas of bronchiolization of alveoli.

Diagnosis: Pleural mesothelioma, sarcomatous type, with metastases.

15057989

B-7

03656893

Hamster No. 82-5. K 545 days.

Depots are seen between all lobes of right lung and between right lung and chest wall. Pleural adhesions extend over all lobes of right lung and over left lung. Incisions of chest organs after fixation show numerous $\frac{1}{2}$ to 1 mm opaque white areas (presumably depots) embedded in glistening gray pleural adhesions. These adhesions are up to 2 mm thick.

A firm gray tumor $22 \times 18 \times 12$ mm is noted anteriorly on the left side. There is an enlarged node (8 mm^3) at base of neck on left. There is an enlarged mesenteric node ($17 \times 7 \times 5$ mm). Adrenal whitish yellow and enlarged 3 times normal.

Histopathology: A pleural adhesion contains macrophages filled with crystalline fibers. No "asbestos bodies." No giant cells. Visceral mesothelium is hyperplastic. Extending along and invading lung and heart is a tumor composed of large plump cells with large vesicular nuclei. Occasional mitoses. In some areas, cells of this tumor are elongated and occasionally multinuclear. A mediastinal node is replaced by large plump tumor cells with large vesicular nuclei, many in mitoses. Mesenteric nodes show hyperplasia. An adenoma in cortex of adrenal measures 3×2 mm.

Diagnosis: Pleural mesothelioma, sarcomatous type, with metastasis. Adrenal cortical adenoma.

15057990

B-8

Hamster No. 16-5. K 570 days.

03656894

Depots extend from right costophrenic angle almost to left costophrenic angle along anterior thoracic surface of diaphragm. Other depots extend between right lower and right middle lobe. A small (2 mm) depot is noted on right upper lobe. Five small (1 to 2 mm) depots lie along posterior surface of left lung. Pleural adhesions are seen over all lobes of right lung and over most of the surfaces of the left lung. There is nothing to suggest intrathoracic tumor:

In the subcutaneous tissue over right thorax, there is a 33 x 25 x 20 mm mass. Right axillary nodes are enlarged (10 x 6 x 4 mm). Mesenteric node is enlarged (16 x 10 x 5 mm). Spleen is enlarged (48 x 9 x 4 mm).

Histopathology: Tumor in subcutaneous tissue and in nodes is histiocytic lymphoma. There are crystalline fibers in lung parenchyma.

Electron microscopy on tumor: Cells with small foci resembling basement membrane. No junctions, no microvilli, no cytoplasmic filaments, no C particles.

Diagnosis: Lymphoma, histiocytic type.

15057991

B-9

03656895

Hamster No. 68-5. K 584 days.

Lungs are bound to each other with thin adhesions; depots thinly spread throughout. Heart is flabby and enlarged. In the position of the right adrenal there is a tumor (22 x 20 x 18 mm) with hemorrhagic and necrotic areas. Tumor is adherent to liver, which is slightly yellow. Kidneys are pale and pitted. Left salivary glands are slightly red and prominent. There is a polyp 2.5 x 2 x 2 mm on left nostril.

Histopathology: Lungs show some pneumonia, depots and adhesions. Tumor in adrenal cortex is composed of large cells with large vesicular nuclei. Cells are very regular in size and shape. Mitoses are rare.

Diagnosis: Adenocarcinoma, right adrenal cortex.

15057992

B-10

03656896

Hamster No. 3-5. K 588 days.

A tumor 15 x 14 x 12 mm lies in subcutaneous tissue over right ribcage in region of injection site. In a 3 x 2 mm area, the tumor extends between ribs and into right pleural space. All lobes of right lung are thinly adherent to each other and to chest wall. Left lung is adherent to chest wall, mediastinum and diaphragm. There are scattered depots in right and left pleura. Other organs not remarkable.

Histopathology: The tumor is composed of irregular spindle cells with much mitotic activity. It invades muscle.
Lungs: dense fibrous plaque.

Diagnosis: Spindle cell sarcoma.

Electron microscopy: Atypical nuclei. Cytoplasmic filaments. No identifiable basement membranes or virus particles. No desmosomes or microvilli are seen.

15057993

B-11

03656897

Hamster No. 78-5. K 600 days.

Lungs have fairly extensive, thin adhesions to each other and to chest in some areas, depots, a fish scale pleural plaque on right lower lobe and a tiny gray area on pleura of left lung. The thyroid is enlarged and firm (4 x 3 x 3 mm). Other organs NR.

Histopathology: Lung: Adenomatous proliferation in one area, with granuloma, fibrosis and bone formation. Other organs NR.

Thyroid: Medullary carcinoma with amyloid stroma.

Diagnosis: Thyroid Carcinoma.

Electron Microscopy: Cells from liver, kidney and mesenteric node were not remarkable. No virus particles were seen. These tissues were examined to learn whether normal hamster tissues might contain virus particles comparable to those remarked in certain tumors in this experiment.

15057994

B-12

Hamster No. 79-5. K 600 days.

03656898

Pleural adhesions over medial surfaces of right and left lobes of lungs. Depots also seen on medial pleural surfaces, with one small depot subcutaneously on right side at injection site. Liver has cysts. Left adrenal is enlarged to three times normal size and is white. Other organs not remarkable.

Histopathology: Lungs show depots, adhesions, granulomas with bone formation in some areas. There are large areas of fibrosis and bronchiolization. Some granuloma in a lymph node.

No tumor. .

Liver has small, benign cysts. Kidney shows amyloid and protein casts. Adrenal has a benign cortical adenoma.

Diagnosis: Adrenal cortical adenoma.

Electron Microscopy: Cells from liver, right kidney and mesenteric node were not remarkable. No virus particles like those seen in tumors in this experiment were found.

15057995

B-13

Hamster No. 88-5. K 600 days.

03656899

Lungs are thinly adherent to visceral pleural surfaces with depots seen in these areas. Bullae of emphysema bulge right middle lobe and left lung. Kidneys and adrenals slightly pale.

Histopathology: Lungs show depots and adhesions with areas of emphysema and atelectasis. There is a pinched off segment of lung with bronchiolization and mesothelial proliferation, but no tumor.

Kidney has plus one amyloid. Adrenal cortex has a very small benign adenoma.

Diagnosis: Adrenal cortical adenoma.

15057996

B-14

03656900

Hamster No. 39-5. K 601 days.

Lungs are moderately adherent to each other and part of chest; depots. Right lower lobe has area of atelectasis. Spleen has a 0.5 mm spot; kidneys are very pale. Right adrenal is large (12 x 10 x 7 mm) and appears to be tumorous. Left adrenal is three times normal and spotted.

Histopathology: Kidneys, amyloid and proteinuria. 1 Adrenal has hemorrhage and necrosis, other has Adrenal carcinoma. Liver: amyloid and hemorrhage. Spleen: amyloid. Lungs: Atelectasis and fibers.

Diagnosis: Adrenal adenocarcinoma.

15057997

B-15

Hamster No. 56-5. K 602 days.

Pleural adhesions bind all lobes of lungs to each other and chest wall. Depots seen scattered over most pleural surfaces. Liver has a few cysts. Kidneys are pale. Left adrenal enlarged to three times normal size and has pale areas.

03656901

Histopathology: Lungs show adhesions, depots and granulomas. Near the heart is fibrous tissue with bone in it.. A focus in lung has giant cells and fibers. Liver has a thickened capsule, giant cells and brown areas. Kidney has amyloid and protein. Adrenal has a benign adenoma in the cortex.

Diagnosis: Adrenal cortical adenoma.

15057998

B-16

Hamster No. 62-5. K 602 days.

Thin pleural adhesions bind most lobes to each other and chest wall, except below right lower lobe. Depots are widespread. Liver is slightly enlarged, and the lobes are rimmed with white. Kidneys look like early nephrosclerosis. Right adrenal is yellowish. Left adrenal is 6 x 4 x 4 mm, hemorrhagic and has a translucent area.

03656902

Histopathology: Lungs have adhesions, depots, granulomas and atelectasis. Liver contains amyloid. Kidney also has amyloid. Adrenal has hemorrhagic areas and a benign adenoma in the cortex.

Diagnosis: Adrenal cortical adenoma.

15057999

B-17

Hamster No. 59-5. K 602 days.

Pleural adhesions bind most of lung surfaces to one another and a good part of chest wall. Depots seen on medial surfaces.

Right middle and right lower lobes have hemorrhagic areas.

Kidneys show question of early nephrosclerosis. Right adrenal is spotted. Left adrenal is enlarged to three times normal size and has a translucent light area.

03656903

Histopathology: Lungs have adhesions, depots, granulomas and giant cells. There are areas with hemorrhage and congestion. Kidney has amyloid and protein. Adrenal has a benign adenoma in cortex.

Diagnosis: Adrenal cortical adenoma.

15058000

Page A-1

APPENDIX A.

GROUP 1.

03656S04

Hamster No. 35-4. 245 days.

Both right and left lungs are widely adherent to parietal pleura by extensive diffuse thin adhesions. Pericardial cavity obliterated by diffuse, thin adhesions. There are depots in right costophrenic angle and between left lung and heart. Mediastinal nodes are enlarged ($15 \times 9 \times 8$ mm) and firm. In right costophrenic angle is a reddish gray mass $32 \times 23 \times 26$ mm densely adherent to base of right lower lobe. This mass depresses and invades diaphragm. Contiguous with the main tumor is a 10 mm hemispherical mass that bulges through and appears to have perforated diaphragm posteriorly on left. In right lateral lobe of liver, a 6 mm pinkish gray hemispherical mound bulges capsule. There is an enlarged ($9 \times 5 \times 4$ mm) left pararenal node. There is a 2 mm discoid pinkish gray nodule beneath capsule at lower pole left kidney. There is a prominent ($10 \times 5 \times 4$ mm) hemorrhagic mesenteric node. Two spherical white nodules (2 mm, 3 mm) lie in mesentery. Other organs not remarkable.

Histopathology: There are dense adhesions between lung and diaphragm with crystalline particles and foreign body reaction. A tumor composed of spindle cells with large vesicular nuclei and prominent mitotic activity surrounds and compresses lung. There are metastases in pulmonary vessels, mediastinal node, liver.

Diagnosis: Pleural mesothelioma, sarcomatous type with metastases.

15058001

A-2

03656905

Hamster No. 43-4. K 361 days.

A mass 24 x 20 x 17 mm lies in chest below heart and left lung. This mass bulges the diaphragm but does not invade the peritoneal cavity. In both right and left pleural spaces there are many scattered depots of injected material on pleural surfaces with some adhesions between lobes of lung and between visceral and parietal pleura. There is an enlarged hilar node.

Histopathology: Compact masses of elongated cells with large vesicular nuclei. Many mitoses. Some of the tumor cells have abundant cytoplasm, some are multinuclear. Metastasis in hilar node.

Diagnosis: Pleural mesothelioma, sarcomatous type with metastasis.

15058002

A-3

Hamster No. 44-4. X 375 days.

03656506

Inflammation of intestines. Large depots of injected material on pleural surfaces of both right and left lungs, but only a few delicate pleural adhesions. Left adrenal enlarged.

Histopathology: Lungs: Fine pleural adhesions. On pleural surfaces there are depots with birefringent fibers in multinucleated giant cells. Adrenal: Benign cortical adenoma.

Diagnosis: Cortical adenoma, left adrenal.

15058003

A-4

Hamster No. 52-4. 413 days.

03656907

Scattered depots in right and left pleural spaces. Pleural adhesions on anterior and posterior surfaces right lung, anterior surface left lung. On posterior surface left lung, a 5 mm mass bulges the pleura.

Histopathology: Mass in left lung: cuboidal, sometimes secretion-containing, cells in a single layer line cystic spaces containing a secretion.

Diagnosis: Mucus adenocarcinoma, left lung.

15058004

A-5

Hamster No. 67-4. K 508 days.

03656908

Subcutaneous edema. Liver very enlarged. Right adrenal enlarged twice normal size. Left adrenal and left kidney each show 1 mm nodule. In both right and left pleural spaces, there are depots and pleural adhesions.

Histopathology: Lungs with pleural adhesions and depots containing fibers. Kidney: not remarkable. Adrenal: adenoma in cortex.

Diagnosis: Cortical adenoma, left adrenal.

15058005

A-6

Hamster No. 8-4. K 514 days.

There is much subcutaneous edema. Heart is enlarged and flabby. There are depots and many adhesions in both right and left pleural spaces. A firm mass 10 x 8 x 6 mm lies in right costophrenic angle. Liver is engorged and has a yellowish-gray area.

03656909

Histopathology: Tumor is composed of spindle cells with prominent mitotic activity. Liver: congestion.

Diagnosis: Pleural mesothelioma, sarcomatous type.

Electron microscopy of tumor: Nuclear irregularity and large nucleoli. Some cells have tight junctions (TJ) and/or desmosomes (D), microvilli (MV), basement membranes (BM), cytoplasmic filaments (F). C particles are noted in endoplasmic reticulum.

15058006

A-7

Hamster No. 26-4. K 570 days.

Depots and adhesions in both right and left pleural spaces. A large mass spreads in all directions from right costophrenic angle and measures 26 x 16 x 13 mm. Other tumor nodules extend beneath heart and up over right upper lobe (RUL) lung. The mass bulges but does not appear to penetrate the diaphragm. Tumor nodules noted in omentum, stomach and peritoneum. The blood is milky. 03656910

Histopathology: Tumor composed of spindle shaped cells. There is invasion of muscle. There are metastases to peritoneum and mesentery.

Diagnosis: Pleural mesothelioma, sarcomatous type with metastases.

15058007

A-8

Hamster No. 79-4. K 577 days.

Massive subcutaneous edema. Liver: 4 times normal size, splotchy purple and yellowish areas. Bloody peritoneal exudate. Spleen slightly enlarged. Kidneys slightly yellowish. Adrenals twice normal size, brownish red. Heart enlarged. Thrombus in left cardiac atrium.

03656911

Lungs: all lobes bound to each other, to chest wall, diaphragm and pericardium by diffuse pleural adhesions. There are depots in both right and left pleural areas. Mediastinal nodes prominent and red.

Neck organs: Cervical nodes are red and enlarged. There is a 17 mm spherical yellowish red mass that on incision is seen to be a solid soft yellowish tumor with extensive areas of hemorrhage.

Histopathology: Lungs: depots and adhesions, no tumor. Thrombus in wall of cardiac atrium. Liver: hemorrhagic. Kidneys: not remarkable. Adrenal: hemorrhage and amyloid. In adrenal cortex there is a lesion resembling adenoma but mitotic activity indicates malignancy.

Neck organs: lymph nodes not remarkable. Tumor is in a salivary gland. It is a solid tumor composed of large rounded cells with abundant faintly basophilic cytoplasm and large vesicular nuclei with occasional mitoses.

Diagnosis: Thrombus, left cardiac atrium.
Congestive failure.
Bilateral pleural adhesions.
Carcinoma, salivary gland.
Carcinoma, adrenal cortex.

15058008

A-9

Hamster No. 57-4. 597 days.

Intussusception of colon into rectum. Kidneys pale.

Adrenals pale and double the normal size. Lungs: depots and adhesions in both right and left pleural spaces. Fish scale plaque on right lower lobe (RLL). Firm, translucent 7 mm lesion on rib cage to left of sternum.

03656912

Histopathology: Pleural and pericardial adhesions.

Depots of fibers in both. Mediastinal nodes contain many fibers in lengths shorter than those seen in depots. There is a calcified pleural plaque. A section of muscle shows bones with marrow cavities (ribs). Seated on this muscle is a tumor composed of long, thin cells with long, thin nuclei. Mitoses are not seen. Cells of this tumor are notably regular in size and shape. They grow between muscle bundles but do not invade muscle fibers.

Diagnosis: Pleural mesothelioma, fibrous type.

15058009

C-1

Appendix C. Group 3.

03656913

Hamster No. 79-3. 204 days.

Had been operated on to remove what had been thought to be blood clots under skin on back. At operation, dark areas in lungs could be seen through rib cage. Animal recovered from operation, but died next day. At autopsy, lungs found studded with at least 40 black discoid lesions 1 - 5 mm in diameter. In right axilla, there is a black mass like those removed the previous day. Hemorrhage in stomach.

Histopathology: Malignant melanoma in fibrous fatty tissue and muscle with 2 nodules apparently lymph nodes; one with residual lymphatic architecture, the other totally replaced by identical tumor (malignant melanoma). Lungs have multiple metastatic nodules of same tumor. Stomach with hemorrhage, no tumor.

Diagnosis: Malignant melanoma, with metastases.

15058010

C-2

Master NO. 47-3. 414 days.

Intestines congested showing evidence of enteritis. Lungs
and organs not remarkable, except for the left adrenal which
as white areas that may be tumorous. 03656514

Histopathology: There is a benign adenoma in cortex of
adrenal.

Diagnosis: Adrenal cortical adenoma.

15058011

C-3

Hamster No. 81-3. K 525 days.

Hamster found moribund, with a red and swollen anus, and had lost weight in the last three months with significant loss of 72 grams in last two weeks.

03656915

Lungs and heart not remarkable. Kidneys are pitted. Adrenals enlarged. The stomach has numerous hemorrhagic ulcers and craters in both squamous and glandular chambers.

Histopathology: Several ulcers in glandular chamber of stomach. In forestomach there is an area with prominent epithelial downgrowths not associated with an ulcer.

Diagnosis: Early squamous cell carcinoma, forestomach.

15058012

C-4

0365616

Hamster No. 9-3. 581 days.

18 x 13 x 13 mm mass in area of right adrenal. 2 mm nodule in left adrenal. Right axillary nodes enlarged 16 x 8 x 6 mm and 12 x 6 x 6 mm. Infection on belly and in seminal vesicles.

Lungs and other organs not remarkable.

Histopathology: Large tumor of adrenal (adenocarcinoma) with a variety of different areas as follows: 1) large eosinophilic cells with many mitoses; 2) smaller eosinophilic cells with dark pyknotic nuclei; 3) small cells with large nuclei and prominent mitotic activity; 4) cells with considerable eosinophilic cytoplasm and very bizarre nuclei; 5) areas of necrosis.

In the other adrenal there is a small benign adenoma with eosinophilic rather regular cells with moderately large vesicular nuclei like the cells in (1), above, but no mitotic activity.

The right axillary nodes are almost completely replaced by the adrenal adenocarcinoma.

Skin: inflammation. No tumor.

Amyloid in liver and kidney.

Diagnosis: Adenocarcinoma, right adrenal, with metastases.

Adenoma, left adrenal.

15058013

C-5

03656917

Hamster No. 6 - 3. K 601 days.

Left adrenal has area $4 \times 3 \times 2$ mm which looks like tumor.
Spleen slightly enlarged and dark ($46 \times 7 \times 3$ mm). Spot in liver.

Histopathology: Adrenal adenoma with moderate amount
mitotic activity. Liver: nodules with increased mitotic activity -
early hepatoma. Spleen: congested.

Diagnosis: Hepatoma and benign adrenal adenoma.

15058014

C-6

Hamster No. 17-3. K 601 days.

03656918

Left adrenal enlarged 10 x 8 x 7 mm. Lungs and other organs not remarkable.

Histopathology: Tumor composed partly of eosinophilic cytoplasm, and partly of nests of small cells with little cytoplasm. Mitoses seen in both types of cells. This is an adrenal cortical carcinoma with differentiated and undifferentiated areas.

No virus particles seen.

Electron microscopy on tumor: Undifferentiated tumor.

Diagnosis: Adenocarcinoma, left adrenal.

15058015

C-7

Hamster No. 21 - 3. K 602 days.

Left adrenal enlarged three times normal (6 x 5 x 5 mm).

Kidneys pitted and with spots.

03656319

Histopathology: Adrenal adenocarcinoma.

Diagnosis: Adrenal adenocarcinoma.

15058016

C-8

03656920

Hamster No. 41-3. K 602 days.

7 x 6 x 4 mm mass in spleen. 6 x 5 x 5 mm cyst in liver.

Lungs and other organs not remarkable.

Histopathology: Nodule in spleen is fibrotic. No mitotic activity or pleomorphism. No follicles. It is a benign tumor. Multilocular cyst in liver is lined by cuboidal epithelium composed of cells that are regular in size and shape.

Diagnosis: Splenoma.

15058017

C-9

03656921

Hamster No. 48-3. K 602 days.

Lungs boggy, some pleural fluid. Kidneys pale. Left adrenal slightly enlarged and spotty.

Histopathology: Kidney: small amount of protein casts in tubules. Some amyloid in glomeruli. Adrenal: small cortical adenoma (benign). Eosinophilic cells. Regular nuclei.

Diagnosis: Adenoma, left adrenal.

15058018